

IN THE CLAIMS

Please amend the claims as follows:

Claim 1 (Currently Amended): A method for producing a heterologous RNA of interest, ~~the method~~ comprising:

(1) transforming ~~the~~ mitochondria of a yeast cells lacking mitochondrial DNA with a mitochondrial transcription vector that comprises at least one copy of the DNA encoding said heterologous RNA of interest under the control of regulatory element(s) for mitochondrial transcription, and a mitochondrial transformation reporter gene or a fragment of said reporter gene;

(2) identifying ~~the~~ a yeast mitochondrial transformants that ~~have~~ has incorporated the DNA of interest;

(3) culturing the yeast mitochondrial transformants selected in (2);

(4) isolating the mitochondria from the yeast mitochondrial transformants obtained in (3), and

(5) extracting and purifying the heterologous RNA of interest from said mitochondria.

Claim 2 (Currently Amended): The method ~~as claimed in~~ of claim 1, wherein said yeast cells lacking mitochondrial DNA ~~are~~ is a  $\rho^0$  cells.

Claim 3 (Currently Amended): The method ~~as claimed in~~ of claim 1, wherein said cells lacking mitochondrial DNA ~~are~~ is obtained from a  $\Delta SUV3$  or  $\Delta DSS1$  strain.

Claim 4 (Previously Presented): The method ~~as claimed in~~ of claim 1, wherein said cells lacking mitochondrial DNA comprises a chromosomal copy of a gene encoding an exogenous RNA polymerase and includes a mitochondrial targeting sequence.

Claim 5 (Currently Amended): The method ~~as claimed in~~ of claim 1, wherein said DNA encoding the RNA of interest is under the control of a promoter and a transcription terminator that are functional in yeast mitochondria.

Claim 6 (Currently Amended): The method ~~as claimed in~~ of claim 1, wherein said mitochondrial transformation reporter gene is a gene encoding one of the proteins of a yeast respiratory chain.

Claim 7 (Currently Amended): The method ~~as claimed in~~ of claim 1, wherein said mitochondrial transcription vector comprises the sequence of an origin of replication of the mitochondrial DNA.

Claim 8 (Currently Amended): The method ~~as claimed in~~ of claim 1, wherein the transformation according to (1) comprises the adsorption of said mitochondrial transcription vector onto metal microprojectiles and the projection of said microprojectiles onto said cells.

Claim 9 (Currently Amended): The method ~~as claimed in~~ of claim 1, wherein (1) comprises the cotransformation of said yeast cells with said mitochondrial transcription vector and a vector that is replicative in yeast; and that comprises a nuclear selection marker.

Claim 10 (Currently Amended): The method ~~as claimed in~~ of claim 9, wherein said nuclear marker is an auxotrophic marker of said transformed cells.

Claim 11 (Currently Amended): The method ~~as claimed in~~ of claim 1, wherein (2) comprises:

(a<sub>0</sub>) crossing the yeast mitochondrial transformants obtained in (1) with a yeast tester strain of *rho*<sup>+</sup> mit<sup>-</sup> type,

(b<sub>0</sub>) identifying ~~the~~ a mitochondrial transformants which, once crossed, gives a diploid cells capable of growing on a non-fermentable medium, and

(c<sub>0</sub>) repeating said crossing until isolated yeast colonies identified as being mitochondrial transformants carrying the mitochondrial transformation vector are obtained.

Claim 12 (Currently Amended): The method ~~as claimed in~~ of claim 1, wherein (1) comprises ~~the~~ cotransformation of said yeast cells with said mitochondrial transcription vector and a vector that is replicative in yeast[[,]] and that comprises a nuclear selection marker, and

wherein (2) comprises:

(a<sub>1</sub>) a first selection or preselection of the yeast cells by means of said nuclear marker, by culturing in an appropriate medium, and

(b<sub>1</sub>) a second selection from the yeast cells selected in (a<sub>1</sub>), which comprises:

crossing the yeast mitochondrial transformants obtained in (1) with a yeast tester strain of *rho*<sup>+</sup> mit<sup>-</sup> type,

identifying ~~the~~ a mitochondrial transformants which, once crossed, gives a diploid cells capable of growing on a non-fermentable medium, and

repeating said crossing until isolated yeast colonies identified as being mitochondrial

transformants carrying the mitochondrial transformation vector are obtained.

Claim 13 (Currently Amended): The method ~~as claimed in~~ of claim 1, wherein the isolation of the mitochondria, in accordance with (4) of the method, comprises lysis or grinding of said cell(s), and then at least two centrifugation steps, at speeds preferably of between 750 g and 12,500 g, and recovery of the final centrifugation pellet.

Claim 14 (Previously Presented): The method of claim 1, wherein (5) comprises:  
eliminating the contaminating nucleic acids in the presence of appropriate buffers, the first buffer comprising at least one divalent ion-chelating agent, and the second buffer comprising an RNase and, optionally, a DNase,

lysing the mitochondria in the presence of at least one detergent and a divalent ion-chelating agent and within a pH range of between 7 and 8, and  
isolating and purifying the RNA of interest.

Claims 15-20 (Cancelled)

Claim 21 (Previously Presented): A method for producing a heterologous RNA of interest comprising:

(1) transforming the mitochondria of a yeast cell lacking mitochondrial DNA with a mitochondrial transcription vector that comprises at least one copy of the DNA encoding said heterologous RNA of interest;

(2) identifying a yeast mitochondrial transformant that has incorporated the DNA of interest;

(3) culturing the yeast mitochondrial transformant selected in (2);

(4) isolating the mitochondria from the yeast mitochondrial transformant obtained in (3), and

(5) extracting and purifying the heterologous RNA of interest from said mitochondria.

Claim 22 (Previously Presented): The method of claim 21, wherein the DNA encoding the heterologous RNA of interest is under the control of at least one regulatory element for mitochondrial transcription.

Claim 23 (Previously Presented): The method of claim 21, wherein said mitochondrial transcription vector comprises a mitochondrial transformation reporter gene.

Claim 24 (Currently Amended): ~~The method of claim 21~~

A method for producing a heterologous RNA of interest comprising:

(1) transforming the mitochondria of a yeast cell lacking mitochondrial DNA with a mitochondrial transcription vector that comprises at least one copy of the DNA encoding said heterologous RNA of interest;

(2) identifying a yeast mitochondrial transformant that has incorporated the DNA of interest;

(3) culturing the yeast mitochondrial transformant selected in (2);

(4) isolating the mitochondria from the yeast mitochondrial transformant obtained in (3), and

(5) extracting and purifying the heterologous RNA of interest from said mitochondria, wherein said mitochondrial transcription vector comprises a fragment of a mitochondrial transformation reporter gene that is not transcribed.